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CHAPTER 14

# Interaction of nitric oxide and external calcium fluctuations: A possible substrate for rapid information retrieval

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## Synapses, the circuit metaphor, and the role of spatial volume in neural processing

Traditional accounts of neurotransmission have described signals passing from one neuron to the next across a synapse, evoking images of wires, nodes, and circuit elements. From this vantage point, the individual synapse has dominated our concept of neurotransmission. In this decade, our conception of neural communication has been broadened by evidence that certain molecules may act as rapid volume signals, which diffuse freely in three dimensions to act throughout local regions of neural tissue. One such spatial signal, nitric oxide, has been implicated in learning, long-term potentiation at single synapses, blood flow control, neurotoxicity, activity-development patterning of synaptic connections, and ongoing control of synaptic transmission.

Nitric oxide (NO) is a non-polar free radical that can readily diffuse through tissue. In neural tissue, NO is known to be made on demand by at least two isoforms of a calcium-dependent enzyme, nitric oxide synthase (NOS). Its capacity to diffuse through cell membranes permits changes in NO production to be felt throughout a diffusion-defined domain. This framework departs from the circuit metaphor described above. In particular, it suggests that spatial volume may be an important parameter for the effects mediated by NO.

NO has a number of targets throughout neural tissue, and it is used in a variety of different signaling pathways. For example, it is a powerful stimulator of soluble guanylyl cyclase and ADP-ribosyltransferase. NO is also a potent vasodilator, which represents a form of negative feedback for the action of NO since (deoxy)hemoglobin is a powerful chelator of NO. NO modulates the probability of neurotransmission on short time scales, and is involved in certain forms of long-term change in synaptic function. Many of these signaling functions are discussed at length in other chapters in this volume.

## Transporting coincidence detection through a tissue volume

NO is made in response to calcium fluxes through the *N*-methyl-D-aspartate (NMDA) type of glutamate receptor. NMDA receptors are both ligand- and voltage-gated, suggesting that they act as molecular coincidence detectors: presynaptic spike arrival is reported by glutamate release, and the

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postsynaptic spike is reported by membrane voltage. The enhanced production of NO subsequent to this coincidence detection provides a means to transport this information throughout a surrounding volume of neural tissue. Furthermore, isotropic diffusion of NO establishes a fixed, radially symmetric relationship between space ($r$) and time ($t$):

$$\langle r^2 \rangle = 6Dt$$

where $D$ is the diffusion constant, and $\langle\ \rangle$ represents the expected value operator.

In this paper, we address three domains in which a rapid volume signal could play important roles in neural tissue: (1) activity-dependent development of neural connections, (2) storage of information in volumes of neural tissue, and (3) rapid retrieval of information from volumes of neural tissue.

## Role of NO in activity-dependent refinement of synaptic connections

The idea of a rapidly diffusing, membrane permeant signal was originally examined on theoretical grounds (Gally et al., 1990). In 1991, four groups demonstrated that the inhibition of nitric oxide synthase (NOS, the synthetic enzyme for NO) prevents the induction of NMDA-dependent LTP in the mammalian brain (Bohme et al., 1991; O'Dell et al., 1991; Schuman and Madison, 1991; Haley et al., 1991). Subsequently, Schuman and Madison provided an elegant demonstration that potentiation could be transported between synapses on different neurons through a diffusible messenger (Schuman and Madison, 1994). Recent work by Bonhoeffer and colleagues is consistent with this interpretation (Engert and Bonhoeffer, 1997). NO has also been shown to play an important role in long-term depression (LTD) in the cerebellum (Shibuki and Okada, 1991; Lev-Ram et al., 1995). These experiments give us clear evidence that a rapidly diffusible messenger molecule like NO influences synaptic function by direct communication through volumes of tissue. Such a signal is ideal for mediating activity-dependent patterning of synaptic connections.

Early theoretical work predicted the involvement of volume signaling in the development of neural connections (Gally et al., 1990; Montague et al., 1991). These computational studies demonstrated that a signal like NO could account for a variety of anatomical patterns of synaptic connections. These include clustered connections in the cerebral cortex, the refinement of topographic mappings in the lateral geniculate nucleus and optic tectum, the formation of ocular dominance columns, and the formation of somatotopic mappings in the cerebral cortex. An example is shown in Fig. 1. Some of these predictions are now supported by experimental data. For example, there are now direct demonstrations that NO is involved in segregation of ON/OFF layers in the ferret lateral geniculate nucleus (Cramer et al., 1996) and the refinement of topography in retino-tectal projections in chick (Wu et al., 1994). However, NO does not appear to play a role in ocular dominance shifts in kitten cortex (Reid et al., 1996), nor in the refinement of retinal ganglion cell axons to eye-specific layers in the lateral geniculate nucleus (Cramer et al., 1996). It is not yet understood why some patterns of synaptic contacts require intact NO production to develop normally and others do not. More work is needed to explore the nature of the differences in specific cases.

## The NOS positive scaffold

As well as playing a role during development, NO also modulates ongoing neural processing. For example, NO enhances release of norepinephrine (NE) and glutamate (Glu) from synaptosomes in the cerebral cortex (Montague et al., 1994). The enhanced release is mediated by activation of the NMDA receptor, and is blocked by agents that inhibit nitric oxide (NO) production or remove it from the extracellular space. NO has also been shown to enhance spontaneous presynaptic transmitter release from hippocampal neurons in dissociated cell culture (O'Dell et al., 1991) and striatal slices (Hanbauer et al., 1992). Thus, NO production may link NMDA receptor activation to changes in neurotransmission in surrounding

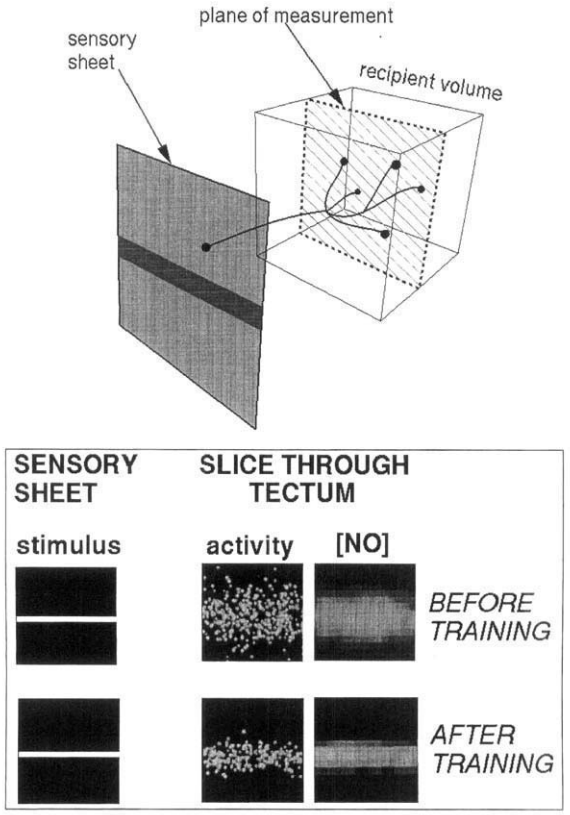



Fig. 1. Example of topographic refinement in a computational model. The development of patterns of synaptic contacts was mediated by a learning rule that stabilized synapses according to the correlation between presynaptic activity and local NO levels. A two-dimensional sensory sheet innervates a three-dimensional tectum with crude initial topography. Elongated waves of activity in the sensory sheet act to refine the topography of the projection. The figure shows a two-dimensional slice through the tectum. This figure is adapted from Montague et al., 1991.

synaptic terminals *whether or not* the synapses are made onto the same postsynaptic neuron. One major consequence of these observations is that volumes of neural tissue may come to act as integrated computational units (Montague and Sejnowski, 1994).

A volume signaling mechanism mediated by NO can play different roles depending on the spatial and temporal scales over which it acts. At small scales, the signal can function rapidly with high specificity. At larger scales, it can act more like a paracrine signal. We have begun to address these issues by examining in detail the scaffold of NOS activity expressed in the mammalian cerebral cortex. The analysis of NOS profiles has been carried out in collaboration with Michael Friedlander.

Three-dimensional distributions of NOS-positive terminals derived from guinea pig and cat cortex were generously provided by M.J. Friedlander. The assumption made here is that the range of NO action in the brain will be a function of the scaffolding of production sites and their temporal pattern of activation. One example distribution from a $93 \times 62 \times 56$ $\mu$m block of tissue is shown in Fig. 2 (top panel). As shown in Fig. 2 (bottom panel), the average first nearest neighbor distance between NOS-positive terminals is 3.9 $\mu$m. Therefore, on average, no point in the cortex is more than $\sim$2 $\mu$m away from a potential NO source. This spacing of NOS positive profiles means that every point in cortex is potentially within about 1 ms of an NO burst, i.e., the temporal width of an action potential (Fig. 3). The same kind of question can be asked in a different way. Fig. 4 shows the percentage of tissue covered by an NO signal as a function of the fraction of NOS positive profiles activated synchronously. It is important to note that we do not yet know how large a burst in NO production actually occurs in functioning tissue.

These conclusions depend on some assumptions that have not yet been tested. One major assumption is that the NOS positive profiles can act as NO sources; there are at least two ways that they could play this role: (1) action potent invading NOS positive profile (on axons or dendrites) could elicit NO production and (2) NO production could result from the nearby release of neurotransmitters. The former case suggests that our estimates of NOS sources may be a gross underestimate since we only analyzed NOS profiles larger than $\sim$0.7 $\mu$m while completely ignoring the heavily stained fibers from which the profiles originate. If these processes constitute an active source of NO, then the estimates we have made fall on the conservative side.

The latter case is intriguing because it suggests that the NOS positive profiles may literally

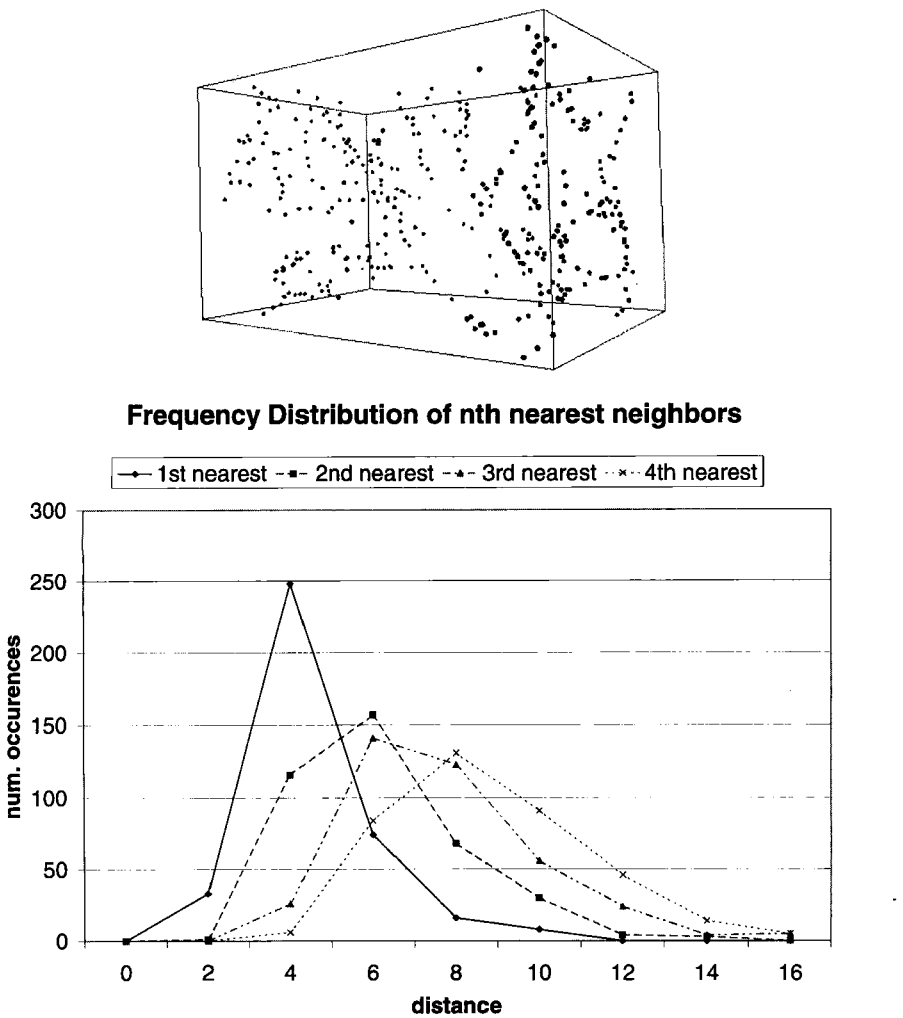



Fig. 2. Three-dimenstional reconstruction NOS positive profiles in a tissue volume. (Top) 92 $\mu$m × 62 $\mu$m × 57 of cat cortical tissue showing the locations of NOS positive profiles (swellings larger than ~0.7 $\mu$m in diameter). (Bottom) Frequency distribution of nearest neighbor distances for the NOS profiles shown in top panel. 1st, 2nd, 3rd, and 4th nearest neighbor distributions (in three dimensions) are shown. The average of first nearest distances is 3.9 $\mu$m. This number is typical for the six tissue blocks analyzed to date.

provide a three-dimensional scaffold from which local signals elicit enhanced NO production. By sensing the neurotransmitters in their locale, the potential NO sources eavesdrop on ongoing activity in the vicinity, decide whether to increase production of NO, and the NO moves rapidly throughout the surrounding volume (increasing transmission probabilities at nearby synapses). Under this model, the ongoing control of NO production acts as a local gain control for transmission throughout a local volume. Experimental data suggests that the fluctuating NO signal does not distinguish one type of synapse from another, i.e., release of glutamate, dopamine, norepinephrine are all enhanced in the presence of an NO burst (O'Dell et al., 1991; Hanbauer et al., 1992; Montague et al., 1994).

The data discussed above paint a picture of positive feedback in NO production, which leads to increased transmitter release, increased calcium

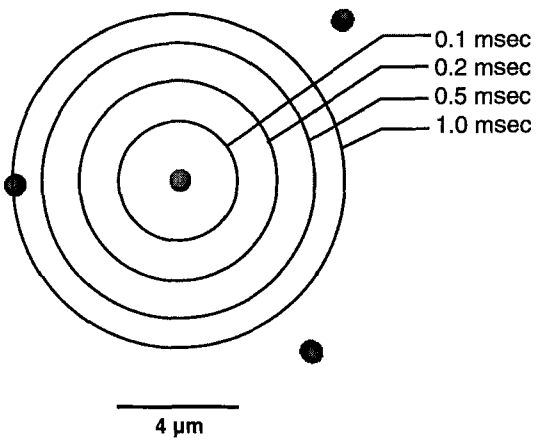



Fig. 3. The relation between root mean square distances and time for NO. The spheres represent NOS positive profiles spaced approximately 4 $\mu$m from their first nearest neighbor. The times and cantered distances are computed from the relationship:

$$r_{RMS} = \sqrt{\langle r^2 \rangle} = \sqrt{6Dt}.$$

entry, and thus increased NO production. This is just one plausible positive feedback cycle. This situation strongly suggests the existence of negative feedback mechanisms on NO production. One negative pathway is the interaction of NO with changes in external calcium levels.

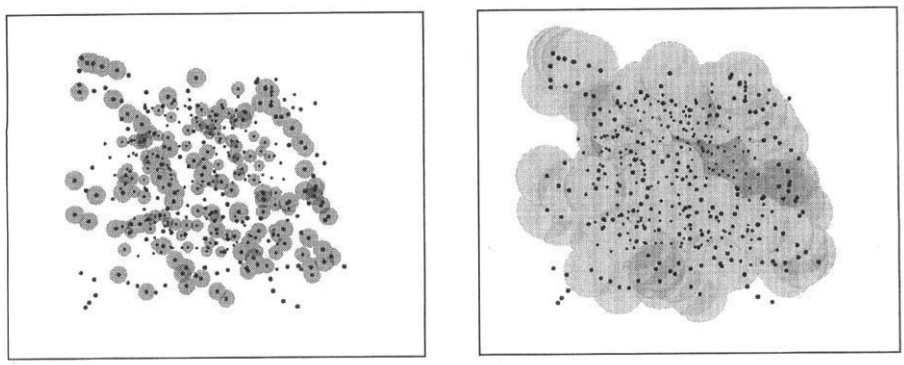
## External calcium fluctuations

We have appealed to NO production as an information-bearing volume signal. We explore here the possibility that interaction between NO and other volume signals will carry functional significance. In particular, we examine the interaction of NO with fluctuations in external calcium. Every *influx* of calcium into intracellular compartments is mirrored by an *efflux* of calcium from the extracellular space. There is a 10 000 : 1 concentration gradient pointing from the extracellular space to intracellular compartments; therefore, any ionic channel permeable to calcium will cause an efflux of calcium from the extracellular space, *i.e.*, net calcium flux through ionic channels is unidirectional. In practice, it is almost impossible to cause calcium currents to reverse. Sodium co-transporters and ATP-dependent pumps are the primary pathways for extruding calcium back into the extracellular space, and these pathways are 2–4 orders of magnitude slower than ionic channels. Thus, an open calcium conductance acts as a rapid sink for external calcium that is replenished on fast time scales by diffusion from surrounding regions of the tissue. These facts along with other considerations have led us to consider fluctuations in external calcium as an information-bearing signal important for neural function (Person et al., 1996; Montague, 1996; Egelman et al., 1998).

In Fig. 5, we show two situations in which external calcium fluctuations are significant. Using a computational model of the extracellular space (Egelman *et al*, 1998) we show the influence of an active presynaptic bouton on external calcium concentrations. The rate of calcium consumption is set to 14,000 calcium atoms per active zone per spike, as reported in the literature by Sakmann and colleagues (Helmchen et al., 1997). For isolated presynaptic terminals, the calcium recovers to baseline rapidly following the arrival of a spike (recovery in less than ~1.5 ms). Different geometrical distributions of calcium sinks will change the recovery time of external calcium. For example, back-propagating action potentials lead to calcium consumption along extended stretches of dendritic membrane (Magee and Johnston, 1997). Fig. 6 shows a small bundle of dendrite segments that are synchronously activated. In the bundle, the recovery time for external calcium is increased dramatically due to the geometry of the synchronous calcium sinks (recovery time increases about 10-fold). In the absence of some preventative mechanism, coincident back-propagating spikes are expected to occur frequently in tissue, even for neurons whose activity is uncorrelated (recall that statistically independent events are quite clumpy in time).

## The interactions of external calcium and N

What is the point of focusing on external calcium fluctuations as a volume signal that interacts with NO? The short answer is that we expect external calcium fluctuations to antagonize increased NO production and its effects. The more interesting answer, discussed in the last section, is that the



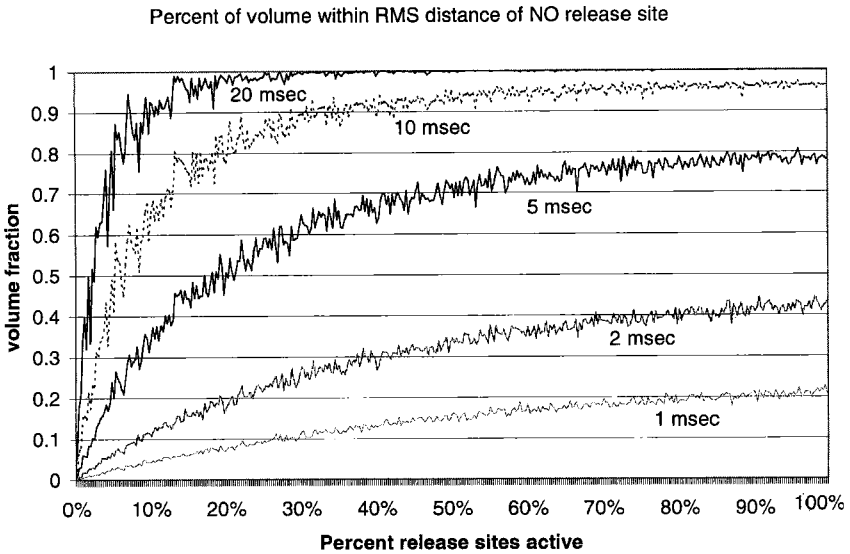



Fig. 4. Time and space tradeoffs for an NO signal. (Top) RMS sphere of NO gas released synchronously from sites defined by a 3-D reconstruction of NOS positive profiles. Left panel shows RMS spheres after 0.2 ms., and right panel shows same spheres 2 ms. (Bottom) This figure shows the fraction of a given volume that has experienced a peak in local NO levels due to synchronous activation of NOS positive profiles from real data. The fraction of the volume covered is plotted against the fraction of NOS profiles activated. Each curve represents a different time after the synchronous burst. By 10 ms, a large fraction of the volume has felt the effects of a synchronous NO burst independent of the exact fraction of NOS profile activated. The percentage volume inside the RMS spheres was calculated using Monte Carlo integration (this accounts for the jagged nature of the curves).

interaction provides a means by which the brain can index different volumes of tissue similar to the way a computer addresses the contents of its memory.

One way for changes in external calcium to antagonize the effects of NO is to decrease the capacity of a tissue volume to make NO. The sequence of events runs roughly as follows. Increased NO production causes increased neurotransmitter release throughout a diffusion-defined domain. This neurotransmitter release includes the release of L-glutamate leading to a large consumption of calcium from the extracellular space in the vicinity. This large consumption results from at least two significant sources: (1) open NMDA receptors, and (2) calcium entry into dendrites due to the effects of back-propagating dendritic spikes caused by the increased level of transmission. The calcium consumption will tend to antagonize the

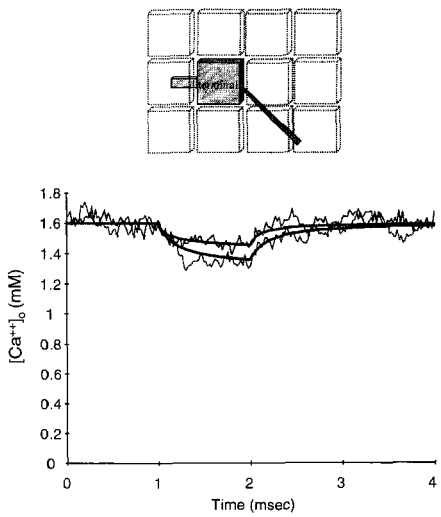



Fig. 5. Calcium consumption by an isolated, active terminal. Using two models of the extracellular space, the decrement in external calcium due to the arrival of a single action potential is shown. Cubic units were packed into a volume. Each cube measured 0.806 $\mu$m along an edge, and yielded the same volume as a 1 $\mu$m diameter sphere (about the size of a bouton or dendritic spine in the mammalian CNS). An explicit Monte Carlo model of passive calcium movement in the extracellular space was used (jagged curves) alongside a discretized model of the extracellular space. Details of the implementation are included in Egelman and Montague (1998). The two curves represent two separate diffusion constants for calcium movement in the extracellular space ($D = 600$ $\mu m^2/s$, and $D = 300$ $\mu m^2/s$). The lower curve is the smaller diffusion constant. The total calcium consumption during the 1 ms action potential was clamped to the value reported in the literature by Sakmann and colleagues (14 000 calcium atoms per spike per release zone). The external calcium fluctuation is brief, and relatively small (~200 $\mu$M for $D = 300$).

effect of NO on further neurotransmitter release. It is well known that small decrements in external calcium can considerably reduce the probability of NT release. The relationship between external calcium concentration and NT is approximately *quadratic*. This has been measured at the squid giant synapse (Katz and Miledi, 1970), the cerebellum (Mintz et al., 1995), and the hippocampus (Qian et al., 1997). It is therefore reasonable to suspect that long, large decrements in external calcium serve to antagonize NO production and its effects.

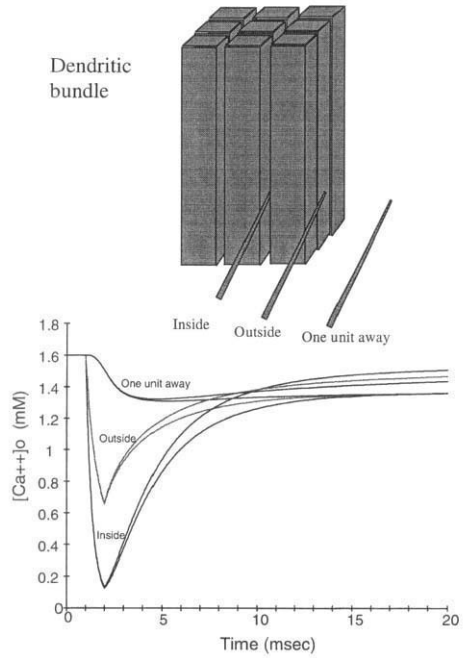



Fig. 6. Bundle of Dendrites. Same computational model as described in Fig. 5. Dendrites are defined by connecting several elementary units like the bouton in Fig. 5. The consumption of each unit area of dendritic membrane was set to 14 000 calcium atoms per spike. Each dendritic segment was 4.03 $\mu$m long. Synchronous activation of the elongated dendritic segments produces elongated calcium sinks whose effects on external calcium superimpose. Two changes result from this different arrangement of calcium sinks: (1) the amplitude of the fluctuation has increased ~6 fold, and (2) the replenishment time has increased from ~7–8 to about 7–10 ms. There are no calcium pumps or co-transporters that would significantly influence either the amplitude or the recovery time. This fact is made clear by the two curves produced for each measurement position. One curve has no calcium pumps present while the faster recovering curve results from the operation of calcium pumps with a 35-ms half-life (pumps are first order in internal calcium concentration). Since this rate is tremendously faster than pumps reported in the literature, the example serves to illustrate that pumps will not change significantly the conclusions.

## Negative feedback at NMDA receptor may act as plasticity gain control

Activation of the NMDA receptor is the first step in the cascade that leads to the production of NO. It is known that NO interacts with the NMDA

receptor to inhibit further influx through the channel (Hoyt et al., 1992; Manzoni et al., 1992; Manzoni and Bockaert, 1993; Tanaka et al., 1993; Akira et al., 1994). NMDA receptor populations may thus be significantly inhibited and possibly silenced under high levels of NO. Taking this possibility further, synapses may be temporarily unable to flux large amounts of calcium, making them refractory to plasticity.

From a biophysical perspective, we see a variety of homeostats in operation. Some event activates NMDA receptors, which leads to increased NO production. The increased NO production indirectly causes further NMDA receptor activation by causing increased release of glutamate from local synaptic terminals. If left unchecked, this cycle could result in two deleterious events: (1) overproduction of NO, which can be neurotoxic under appropriate oxidizing conditions, and (2) large fluxes of calcium into cells, which can lead ultimately to the induction of apoptosis. We have highlighted two negative feedback mechanisms for this positive feedback cycle: (1) NMDA receptor activation turns to a prolonged sink for external calcium, and (2) NO inhibits fluxes through the NMDA receptor thus turning off one primary stimulus for its own production. Below, we consider some computational consequences of these biophysical possibilities.

## Computational consequences of the calcium–NO cycle

### *Push-pull character of NO production and calcium production*

We have already suggested that NO and external calcium tend to move in directions that anatagonize changes in each variable. This situation suggests that the production and removal of NO and external calcium are coupled. This fact can be expressed generally:

$$\frac{\partial n}{\partial t} = f(n,c) + D_n \frac{\partial^2 n}{\partial x^2}, \tag{1}$$

$$\frac{\partial c}{\partial t} = g(n,c) + D_c \frac{\partial^2 c}{\partial x^2} \tag{2}$$

$n(x,t)$ and $c(x,t)$ are functions of time ($t$) and space ($x$) and represent respectively fluctuations of nitric oxide levels $N(x,t)$ and external calcium $C(x,t)$ about their equilibrium values $N_0$ and $C_0$. $f(n,c)$ and $g(n,c)$ are functions which may be non-linear in their arguments. The last term in the equations represents the passive diffusion of fluctuations. There are two particular facts about external calcium and nitric oxide that make these expressions more interesting; they allow a straightforward interpretation of $f$ and $g$ as being proportional to concentration curvatures (in the tissue space) of calcium and nitric oxide. (1) All the nitric oxide is made from enzymes located inside cells where the calcium levels are kept near zero, and (2) ionic channels that conduct calcium always conduct it *out* of the extracellular space. If external calcium goes down, internal calcium goes up. Some of the internal compartments that see this increase contain NOS, and will increase NO production. We translate these remarks into a simple linear equation by noting that a positive curvature in external calcium $(\partial^2 c/\partial x^2 > 0)$ suggests that NO is getting made somewhere; giving us a specific form for $f(n,c)$:

$$\frac{\partial n}{\partial t} = K_c \frac{\partial^2 c}{\partial x^2} + D_n \frac{\partial^2 n}{\partial x^2}. \tag{3}$$

Furthermore, increases in NO production cause a negative curvature in the NO concentration, and are modeled as negatively proportional to the increase in external calcium (NO inhibits calcium fluxes out of the extracellular space thereby raising external calcium levels):

$$\frac{\partial c}{\partial t} = -K_n \frac{\partial^2 n}{\partial x^2} + D_c \frac{\partial^2 c}{\partial x^2}, \tag{4}$$

where $K_c$, $K_n$, $D_c$, $D_n$ are positive constants. We are left with an interesting set of coupled equations for NO and external calcium. These equations can be re-written using a single complex variable $z = c + in$. We do not explore the biological

consequences in this chapter, but this composite variable provides a novel signal that is ideally suited for the storage of important parameters (Montague, unpublished observations).

*Gain control on plasticity*

Our hypothesis is that calcium influx at a synapse (and thus the eligibility for plasticity) can be modulated by the ambient NO concentrations. The NMDA receptor is necessary for certain kinds of plasticity, but makes only minimal contribution to the excitatory postsynaptic potential (EPSP). It is straightforward to imagine transmission continuing unaffected at a synapse while the plasticity is shut down. We propose that ambient levels of NO in the brain may render some fraction of a synapse's NMDA receptor population silent at any given time, independent of the status of glutamate binding to the receptor. Increases or decreases of the NO concentration could modulate a synapse's eligibility for NMDA-mediated plasticity. In this framework, a sufficient rise in local NO concentration results in a greater fraction of inactivated NMDA receptors. Conversely, decreasing NO levels leads increased eligibility for plasticity.

*A biological addressing scheme: Rapid indexing of small volumes of neural tissue*

The possible interactions of external calcium and NO have given rise to several new suggestions about their function in neural tissue. These functions have been described only at the level of biophysical changes – when receptors may operate, when plasticity is on or off, and so forth. There is, however, a computational perspective for the meaning of these physical interactions. We propose that the interaction of external calcium fluctuations and NO provides a rapid indexing scheme used by the brain to locate specific volumes of neural tissue (presumably containing information important for some function).

Our approach here is to view the cerebral cortex as an enormous storage device that acts as a look-up table where the kinetics of the look-up process can implement a variety of functions. For example, in some cortical areas, this look-up amounts to iconic retrieval of information, *e.g.* cells in the inferior temporal cortex that respond to specific faces or views of faces. In other areas, like the visual or somatosensory cortex, the look-up process is equivalent to an information processing algorithm well fitted to some problem domain, *e.g.* the visual problem of detecting the likely presence of a contiguous border that is occluded by overlying objects. In this section, we simply detail how a specific, small volume of tissue could be indexed by a pattern of neural signals (Fig. 7).

Information arrives in a region of tissue, and the job of the biological mechanisms is to retrieve stored parameters in an appropriate amount of time. In some cases, the dynamics of the retrieval itself is the sought-after information, and in other cases, retrieval may take other forms. Here, we only address the issue of indexing the desired locations in tissue given a collection of signals impinging on a region of cortex. One of the computational problems to be solved is the natural trade-off between storage capacity and retrieval time. Greater storage capacity makes more difficult the task of keeping retrieval times short. An unlimited memory is not useful unless the information can be accessed in an appropriate amount of time, and a serial search through large memory is almost never feasible. These kinds of concerns were surely a ubiquitous evolutionary constraint: mobile organisms always had to squeeze the results of neural computations into behaviorally relevant timescales. For example, no matter how much *parallel* processing takes place in the cortex, there is an enormous *serial* processing constraint for choosing which direction to turn next when being chased by a predator. Any computational result has to be ready and available quickly if it is to be useful. This is an extreme example; however, all perceptual tasks for a mobile creature are subject to strict temporal constraints; therefore, the mechanisms employed by the cortex are also subject to these same constraints.

One way to avoid serially searching through a large memory every time a query for information is made is to use clever ways of organizing the information. The cerebral cortex solves the rapid look-up problem in part by clumping similar functions in similar volumes of tissue. This idea is interesting and suggestive, however, it falls short of specifying how specific subelements of the volumes, e.g. specific synapses or their components, could be addressed by a pattern of neural activity arriving in the form of different neurotransmitters.

The computational idea for mediating rapid look-up is outlined below, and followed by our interpretation of a possible neural substrate. Consider the cortex as a storage medium where information (or functions) are clustered in common volumes of tissues. The indexing has multiple stages, and takes advantage of this scheme for organization information (Fig. 7):

*Stage I* (Global query). A global query is issued through the broadcast of a vector of cues. Specific regions match this vector best. These regions are labeled as prototypes – they serve to identify the likely locations of the storage medium containing the desired information or response.

*Stage II* (Prototype generalization). The search is expanded rapidly around a subset of the prototypes taking advantage of the organizational scheme of clustering similar function in nearby regions of the medium.

*Stage III* (Refinement). Generalization about each matching prototype has identified many possible locations, and a more refined analysis is required in these regions. The refinement step executes a rapid, more detailed search only in the regions defined by the domains centered on the prototypes. This identifies even smaller volumes likely to contain the best fit to the global query.

To mark the results, a signal is generated that flags the region of the medium containing the results – the signal means something like 'I am the best fit to the query'.

Notice that Stage I imbeds an interesting assumption into the nature of the storage medium, i.e., the system cannot be sure of the exact location of the information; therefore, a broadcast to many areas must occur. This incurs a cost – the cost in time and space of constructing and distributing the broadcast. The payback for this expense is that the memory can be large, yet the system maintains recall within an acceptable time window. We suspect that in the cortex, this scheme could operate on the scale of less than 10 ms (described below).

The scheme can also be applied recursively. Once the refinement stage has located a set of smaller volumes, the cycle could repeat using these smaller volumes as new prototypes. Iterating these cycles may allow the localization of tissue volumes on the scale of just a few microns. This description has dealt very generally with locating specific volumes in a storage medium. There is a remarkable correspondence in the calcium–NO interaction that could act as a substrate for this multistage process.

The interactions of external calcium and nitric oxide give a mechanism that could solve this problem of looking-up specific volumes of tissue. The neural scheme makes use of some of the facts described earlier:

(1) The NO scaffolding places potential sources within close proximity to every point in the cortex.
(2) NO moves isotropically through tissue and increases transmission events from nearby synaptic terminals.
(3) Increasing transmission events contributes to spike production in recipient neurons, *and* directs back-propagating spikes into the region where the transmission events occurred.
(4) Back-propagating spikes that overlap in time and tissue space cause large decrement in external calcium (Fig. 6).

The neural sequence would occur as follows:

*Global query to a region of tissue*: A vector of transmitters is released across a fairly large region of tissue by patterns of action potentials. Some volumes match best the transmitters and their temporal variations. This locates the prototype matches.

*Prototype generalization*: For some prototypes, NMDA receptor activation occurs and is followed

by a burst in NO production. NO moves rapidly through the surrounding tissue, increases transmission events, and turns down NMDA receptor sensitivity. The diminishment of plasticity serves to insulate the tissue from changing dramatically the contents of the storage location (we view this primarily as a look-up or 'read' mechanism).

*Refinement*: The increased transmission events following the NO burst serve two roles: (1) inactivate A-type potassium channels in dendrites in the region, and (2) provide input to recipient neurons that contributes to spike production. The back-propagating spikes travel into the dendrites, and are directed primarily to those regions where the transmissions occurred (Magee and Johnston, 1997). These spikes are restricted to dendrites; therefore, they represent a search through a domain vastly smaller than the one defined by the NO burst. This *'search-along-wires'* causes large peridendritic fluctuations in external calcium, and sets up physical constraints necessary for implementing the last step (flagging the results).

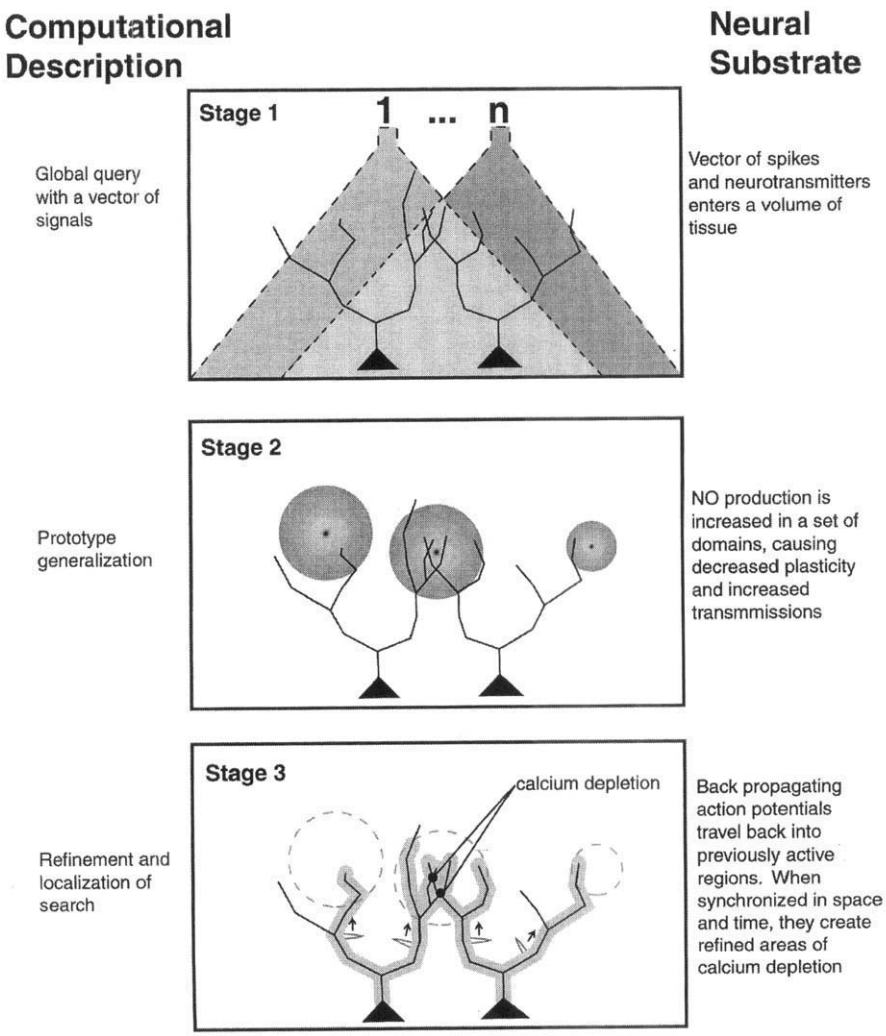



Fig. 7. NO and calcium may implement a rapid addressing scheme in neural tissue. The interaction of NO production and external calcium levels produces a plausible substrate by which patterns of neural activity are used to 'look-up' specific volumes of tissue. Details of the multistage process are given in the text.

*Marking the results*: If the back-propagating spikes overlap sufficiently in time and tissue space, a large and longer lasting fluctuation in external calcium 'flags' a set of much smaller volumes ('here we are'). If no such fluctuation occurs, the system has not located any sufficiently similar information matching the global query. The details of the dendritic structure and patterns of synaptic contact determine whether a given pattern of back-propagating spikes will cause a large fluctuation in external calcium. This means that information could be stored in the relative three-dimensional location of synaptic contacts, and in the relative location of dendritic branches.

## Closing remarks

The recent discoveries about NO diffusion through volumes of tissue place the emphasis of neural encoding not on the single neuron, but rather on the volume of tissue in which the neurons function. Solving the problem of how to address a large parameter space may provide guidance for discovering the biological mechanisms of memory storage and retrieval. We have shown how a novel interaction of NO and external calcium levels can act together to address specific volumes of neural tissue. However, the addressing scheme proposed here is mute to the question of *what* is actually read out. Indeed, we have left this out because understanding the specifics of the read out requires some insight into how the problem domain is encoded in the first place, *i.e.*, we need a better guess at what the read-out should represent. The latter question is quite a general one for all of neuroscience, and remains open to speculation.

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